

# Genetic and Mutational Analyses of a Large Multiethnic Bardet-Biedl Cohort Reveal a Minor Involvement of *BBS6* and Delineate the Critical Intervals of Other Loci

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Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder characterized primarily by obesity, polydactyly, retinal dystrophy, and renal disease. The significant genetic and clinical heterogeneity of this condition have substantially hindered efforts to positionally clone the numerous *BBS* genes, because the majority of available pedigrees are small and the disorder cannot be assigned to any of the six known *BBS* loci. Consequently, the delineation of critical *BBS* intervals, which would accelerate the discovery of the underlying genetic defect(s), becomes difficult, especially for loci with minor contributions to the syndrome. We have collected a cohort of 163 pedigrees from diverse ethnic backgrounds and have evaluated them for mutations in the recently discovered *BBS6* gene (*MKKS*) on chromosome 20 and for potential assignment of the disorder to any of the other known *BBS* loci in the human genome. Using a combination of mutational and haplotype analysis, we describe the spectrum of *BBS6* alterations that are likely to be pathogenic; propose substantially reduced critical intervals for *BBS2*, *BBS3*, and *BBS5*; and present evidence for the existence of at least one more *BBS* locus. Our data also suggest that *BBS6* is a minor contributor to the syndrome and that some *BBS6* alleles may act in conjunction with mutations at other *BBS* loci to cause or modify the BBS phenotype.

## Introduction

Positional cloning has proven successful in the identification of genes for monogenic disorders. However, the presence of genetic heterogeneity often complicates such efforts, particularly for rare recessive disorders. Bardet-Biedl syndrome (BBS [MIM 209900]) exemplifies such instances. BBS is a multisystem autosomal recessive disorder characterized by rod-cone dystrophy, polydactyly, central obesity, hypogonadism, learning difficulties, and renal dysplasia. Other features that vary in frequency include diabetes mellitus, hepatic fibrosis, reproductive abnormalities, endocrinologic deficiencies, short stature, developmental retardation, and speech and behavioral abnormalities (Green et al. 1989; Beales et al. 1999).

The estimated population prevalence varies from 1/13,500 among the Bedouin of Kuwait (Frag and Teebi 1989) to 1/160,000 in Western Europe (Klein and Ammann 1969).

Six BBS loci have been identified to date: *BBS1* on 11q13 (Leppert et al. 1994), *BBS2* on 16q21 (Kwitek-Black et al. 1993), *BBS3* on 3p12-13 (Sheffield et al. 1994), *BBS4* on 15q23 (Carmi et al. 1995b), *BBS5* on 2q31 (Young et al. 1999a), and *BBS6* on 20p12 (Katsanis et al. 2000; Slavotinek et al. 2000). However, only one *BBS* gene has been cloned (*BBS6/MKKS*) (Katsanis et al. 2000; Slavotinek et al. 2000). Furthermore, despite some efforts to assign clinically this variable phenotype to the different genetic loci, data correlating specific phenotypic features with individual *BBS* genes have been inconclusive (Carmi et al. 1995a; Beales et al. 1997; Young et al. 1998). Thus, in the absence of either large families or pedigrees from population isolates, genetic analysis of unrelated families remains the only reliable means of differentiating loci (Carmi et al. 1995a; Beales et al. 1997; Bruford et al. 1997; Young et al. 1998, 1999b; Katsanis et al. 1999; Woods et al. 1999).

Three studies have attempted to assay the distribution of the *BBS* loci (Beales et al. 1997; Bruford et al. 1997;

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Katsanis et al. 1999). *BBS1* accounts for the disorder in 36%–56% of pedigrees, *BBS2* in 24%–27% of pedigrees, and *BBS4* in 32%–35% of pedigrees. Only a single verification of the *BBS3* interval, in a Newfoundland kindred, has been reported previously, bringing the total number of families in which the disorder maps to that locus to two (Sheffield et al. 1994; Young et al. 1998). The existence of *BBS5* on 2q31 (Young et al. 1999a) has yet to be confirmed independently. *BBS6* on 20p12-p13 was also recently identified, and mutations in the McKusick-Kaufman syndrome gene (*MKKS*; Stone et al. 2000) were described for this locus (Katsanis et al. 2000; Slavotinek et al. 2000).

We present here the results of a screen for *MKKS* mutations in 163 pedigrees with BBS, and we appraise the contribution of known BBS loci in the North American/northern European populations with a combinatorial strategy of linkage disequilibrium and haplotype analysis. Unlike the distribution of BBS disease alleles in Newfoundland, where *BBS6* is nearly as common as *BBS1* (Katsanis et al. 2000), linkage to *BBS6* was found in only ~4% of pedigrees from our more diverse cohort. We also report data from North American, European, Turkish, Iraqi, Pakistani, and Indian populations that reduce substantially two of the four critical intervals, potentially rendering them amenable to positional cloning, and we report mapping of BBS to *BBS5* on 2q31 in three families, the first independent evidence supporting the existence of this locus. Finally, we demonstrate, in several pedigrees, the exclusion of all known BBS loci, and we suggest that at least a seventh, yet unmapped, locus exists in the human genome.

## Patients and Methods

One hundred sixty-three BBS pedigrees were screened for mutations in *MKKS*. Fifty pedigrees (27 North American/European and 2 Newfoundland pedigrees for which *BBS1* was excluded through haplotype analysis, and 21 consanguineous pedigrees of Turkish, Iraqi, Pakistani, and Indian origin) were included in linkage analyses. The diagnosis of BBS was based on established criteria in which three of six cardinal features must be present (Beales et al. 1999). In several cases, the diagnosis was ascertained by local physicians and verified through extensive examination of medical records by one or more of us (P.L.B., R.A.L., J.S.G., or P.S.P.).

Blood was obtained, with informed consent, in accord with protocols approved by the appropriate oversight committees at each institution, and DNA was extracted by a salting-out process (Puregene, Gentra Systems). Direct sequencing of *MKKS* was performed as described elsewhere (Katsanis et al. 2000). *MKKS* primers for mutational analysis can be retrieved from the Lupski lab home page. For the genetic analyses, a total of 54 custom-

synthesized (MWG/Sigma-Genosys) fluorescent microsatellite STRPs were typed for each family member: for *BBS1*: *D11S4205*, *D11S1883*, *D11S599*, *D11S449*, *D11S1889*, *D11S4909*, *D11S4946*, *PYGM*, *D11S4945*, and *D11S4944*; for *BBS2*: *D16S411*, *D16S415*, *D16S419*, *D16S390*, *D16S408*, *D16S526*, *D16S3089*, *D16S265*, *D16S3034*, *D16S408*, *D16S3057*, *D16S514*, *D16S503*, *D16S400*, *D16S421*, and *D16S515*; for *BBS3*: *D3S1566*, *D3S1276*, *D3S3634*, *D3S1603*, *D3S1251*, *D3S2419*, *D3S1271*, and *D3S1278*; for *BBS4*: *D15S117*, *D15S153*, *D15S125*, *D15S988*, *D15S814*, *D15S650*, *D15S131*, *D15S204*, *D15S114*, and *D15S205*; for *BBS5*: *D2S151*, *D2S142*, *D2S156*, *D2S2330*, *D2S335*, and *D2S326*; and for *BBS6*: *D20S115*, *D20S851*, *D20S189*, and *D20S186*. Primer sequences were obtained through the Genome Database or the MIT Center for Genome Research. All DNA samples were adjusted to 30 ng/ml, and PCR reactions were performed on an MJR Tetrad or an MWG Primus thermocycler. Products were resolved on an ABI 377 automated sequencer, and alleles were assigned with GENESCAN v2.3 and GENOTYPER v2.1 software (Applied Biosystems). Sequence data were managed on Sequencher (Genecodes Corporation). Linkage analysis was performed, where appropriate, with the LINKAGE package of programs: two-point linkage data were generated with MLINK (assuming a gene frequency of .005 and a penetrance of .9); multipoint analyses were performed with LINKMAP.

## Results

### *Mutation Analysis of MKKS (20p12)*

We sequenced the open reading frame (ORF) of *MKKS* in all 163 pedigrees regardless of their haplotype-inferred locus assignment. In eight pedigrees, we identified missense alterations that segregate with the disease (table 1). These were not present in a minimum of 188 chromosomes from matched control populations. In contrast to previous findings, in which most disease-causing mutations were frameshifts (Katsanis et al. 2000; Slavotinek et al. 2000), all changes identified in this survey resulted in missense alterations that may affect the solubility and/or the structure of the *MKKS* protein (table 1). We found no alterations in either exon 4 or exon 5 of *MKKS*, which suggests that mutations in that region of the molecule may be nonpathogenic, may not result in BBS, or may be too severe to allow viability of the embryo. We did, however, detect three changes in exon 6 that would affect the C-terminus of *MKKS*. Evaluation of the clinical phenotype in these patients did not reveal substantial differences from the other BBS pedigrees, although the numbers are too small to allow meaningful conclusions.

The A242S change that we describe in family NF-B14

**Table 1****MKKS Nucleotide Alterations Found Exclusively in Patients with BBS**

Pedigree	Exon	Alteration	Potential Consequence
NF-B14	3	A242S	Also reported in MKKS family (Stone et al. 1999)
AR-259	3	Q147X	Change to stop codon—truncated protein
AR-301	3	T57A	Potential solubility change
AR-396	3	I32M	Start methionine introduced in good Kozak context
AR-396	3	S235P	Insertion of kink in $\alpha$ helix
AR-127	3	D285A	Potential solubility change
KK-062	6	R518H	Unknown
AR-579	6	C499S	Secondary structure change
AR-017	6	S511A	Potential solubility change

has been shown to cause disease in conjunction with an H84Y mutation. This complex homozygous H84Y/A242S allele results in McKusick-Kaufman syndrome in the Old Order Amish (Stone et al. 2000). We were unable to find the second mutant allele in family B14, but haplotype analysis with microsatellites across the *BBS6* region demonstrated that the two sibs have identical 20p12 haplotypes. Notably, the affected sib in this pedigree is homozygous for markers across the *BBS2* critical interval on 16q21 (fig. 1). The A242S alteration was found once in 142 Newfoundland control chromosomes and in none of 188 North American/European control chromosomes, suggesting either that it is a very rare polymorphism or that its involvement in the disease does not follow a classical model of recessive inheritance.

#### *Distribution of BBS Pedigrees and Delineation of Critical Intervals*

None of the pedigrees included in this study is large enough to generate statistically significant positive LOD scores with any marker. Therefore, with the exception of mutational analysis of *MKKS*, it is not possible, in any sibship, to assign the disorder to a locus by mathematical means. However, given that the known *BBS* loci appear to account for nearly 90% of this syndrome in the North American/European population, it is possible to assign individual loci in some pedigrees when haplotype analysis excluded, in any given sibship, all but one *BBS* locus (Katsanis et al. 1999). Using such assignments, we proceeded to delineate the different *BBS* critical intervals through the identification of ancestral recombinations (29 pedigrees) and/or loss of identity-by-descent (IBD; 21 pedigrees) in families with demonstrated or suspected consanguinity.

#### *BBS2: Chromosome 16*

Through haplotype construction of eight markers spanning the known *BBS2* critical interval, we determined that eight pedigrees were consistent with linkage to 16q21. The haplotypes of all eight kindreds excluded the other five loci, and thus increased the probability of assignment to *BBS2*. The order of markers on chro-

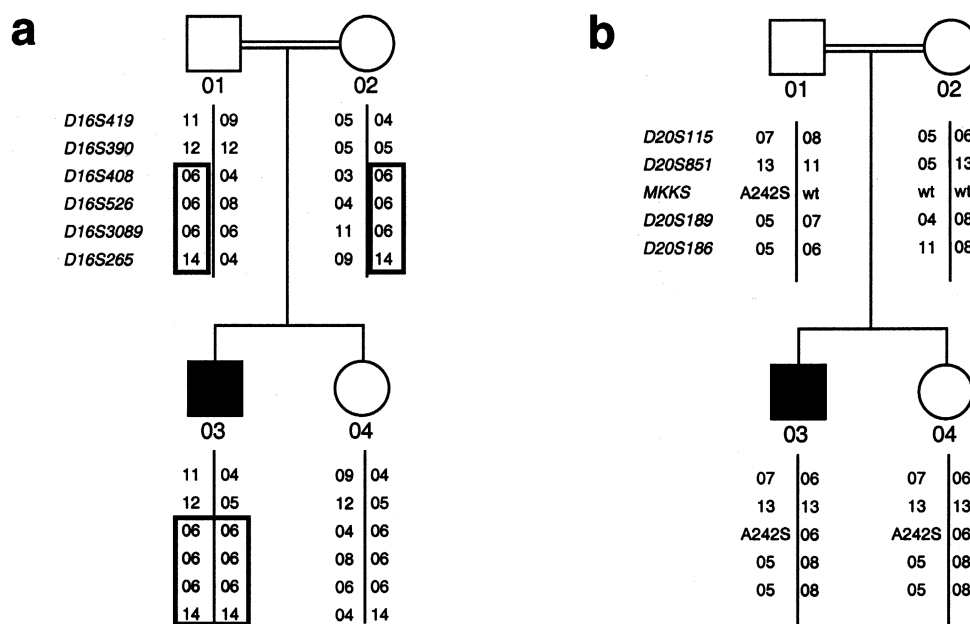
mosome 16 and the relative position of recombinational events or IBD are summarized in figure 2*a*. We detected recombinations in three families: AR-029, AR-083, and PB-005 (fig. 3*a*). In addition, AR-042 demonstrated a region of IBD extending from *D16S408* to *D16S400*. Although this family is not known to be consanguineous, both parents have maternal French-Acadian ancestry, which may imply a founder mutation. The grandpaternal side of the family is less well documented but originates from the US-Canadian border. The other pedigrees compatible with linkage of the disorder to *BBS2* did not contain recombinants across the marker set studied. Taken together, these data suggest that *BBS2* lies between *D16S408* and *D16S3057*, a genetic distance of ~2 cM, which, according to our physical maps of the region, is ~1.5 Mb; loss of IBD in AR-042 at *D16S3057* further supports the distal boundary of this new critical interval (fig. 2*a*). This represents a substantial reduction of the 18-cM critical interval reported elsewhere (Kwitek-Black et al. 1993; Bruford et al. 1997).

#### *BBS3: Chromosome 3*

Using a similar analysis strategy, we identified two pedigrees that were consistent with mapping of the disorder exclusively to the *BBS3* locus. Analysis of these pedigrees for markers in this critical interval revealed the presence of a recombination in AR-201 individual -06 with marker *D3S1566* and a distal recombination in an unaffected individual -04 at marker *D3S1251*, suggesting that *BBS3* most likely lies between these two microsatellites (fig. 3*b*). These data substantially refine the 6-cM critical interval reported elsewhere (Young et al. 1998) and refine the critical interval to between *D3S1603* and *D3S1251*, a distance of 2 cM or 1.1 Mb (fig. 2*b*).

#### *BBS4: Chromosome 15*

Only a single pedigree, AR-287, has a haplotype pattern consistent with linkage to chromosome 15 (*BBS4*) (fig. 3*c*). All three affected individuals share a common haplotype extending from *D15S650* through *D15S205*, a distance of 12 cM. We identified a recombination at



**Figure 1** Haplotype analysis of Newfoundland pedigree B14 with Hsa 16 (a) and Hsa 20 (b) markers from the critical intervals of *BBS2* and *BBS6*. IBD markers from the *BBS2* region are boxed. Note that individuals -03 and -04 have the same haplotypes for the *BBS6* markers tested and carry the same A242S alteration. Individuals -01 and -02 are second cousins.

*D15S814* in individual -03, thus placing the *BBS4* gene distal to that marker. Bruford et al. (1997) narrowed the interval to 2 cM through two recombinants, placing *BBS4* between *D15S131* and *D15S114*; our interval concurs with that of Bruford et al. (1997) (fig. 2c).

#### BBS5: Chromosome 2

Haplotype analysis of all 29 pedigrees suggests that 3 are consistent with linkage to chromosome 2q31. The affected individuals from pedigrees AR-199, AR-273, and AR-082 share haplotypes extending from *D2S142* (cen) to *D2S326* (tel) without recombination (fig. 2d). This interval spans 13 cM or ~7 Mb and does not improve the original published interval derived from a single Newfoundland family described by Young et al. (1999a). Tests for heterogeneity were inconclusive because of the small numbers of pedigrees and their relatively small size. However, if considered together, these three pedigrees generate a two-point LOD score of 2.2. Given that, in these pedigrees, linkage to all the other loci could be excluded, our findings suggest the first independent confirmation of the *BBS5* locus on 2p31, which, as expected, contributes only a small number of *BBS* pedigrees to the syndrome.

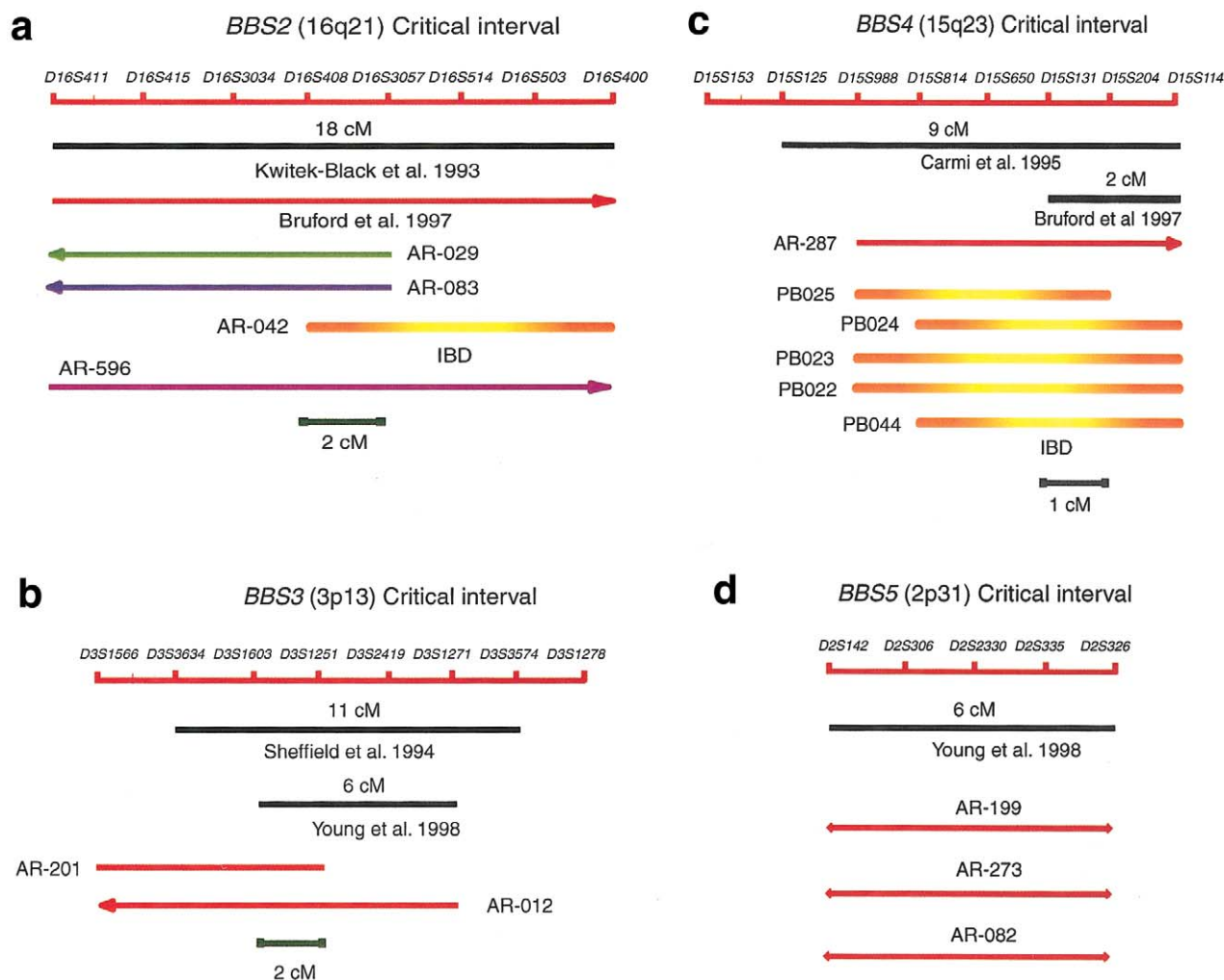
#### BBS6: Chromosome 20

Since we were not able to detect the second mutation in several pedigrees, despite the identification of an alteration that fulfilled all other genetic criteria of patho-

genicity, we analyzed other markers flanking *MKKS*. Of the eight pedigrees containing likely *BBS6* mutations, four pedigrees of North American origin were compatible with linkage to 20p12, two of which we described elsewhere (Katsanis et al. 2000). One pedigree (NF-B14) was expected to show linkage of the disorder to the *BBS2* locus; in the remaining pedigrees, the disorder could not with confidence be excluded from one or more other loci and thus no locus could be assigned, by our criteria. Overall, according to our mutational analysis, 4% of pedigrees from our cohort show linkage of the disorder to *BBS6*.

#### BBS7: Unknown

We performed genetic analyses of 27 pedigrees of North American/European origin and of 2 Newfoundland pedigrees in which linkage to *BBS1* had previously been excluded. Fifteen of these do not show convincing evidence for linkage of the disorder to any of the known *BBS* loci. We were unable to categorically exclude five of them, owing to either small family size or uninformative markers within some critical intervals. The remaining pedigrees ( $n = 10$ ) represent 34% of this group of families, which did not initially show linkage to 11q13 as reported by Katsanis et al. (1999). When the pedigrees with evidence for linkage to chromosome 11q13 and the 4% contribution of *BBS6* are considered in the analysis, the overall proportion of kindreds without linkage falls to 14%, suggesting that one or more *BBS* loci remain unmapped (fig. 4). Detailed analyses showed that, in two



**Figure 2** Schematic diagram of marker order on respective BBS chromosomal regions and the relative positions of recombination events and extent of IBD. Arrowed lines depict extent of linkage, with the blunt end denoting recombination; solid graduated lines indicate regions of IBD.

pedigrees (AR-707 and NF-B6), the disorder could be excluded convincingly from all known loci both through the generation of a LOD score of  $<-2.0$  and by haplotype analysis, confirming the hypothesis that at least one more BBS locus (*BBS7*) exists in the human genome.

#### Non-European IBD Study

We studied 12 Turkish families, 1 Iraqi family, 7 Pakistani families, and 1 East Indian family. Each affected child was the result of a consanguineous union between first-cousin parents; thus, haplotype analysis of BBS-affected individuals should reveal IBD markers over part or all of the critical region. If IBD was absent at any locus, then that family was considered “unlinked” for the purposes of this study. A total of 93 individuals were genotyped with at least three markers spanning the most recently published critical interval at each locus (*BBS1*-

6). Nine families had at least two affected members, 2 families had three affected members, and 10 families had only a single affected member. Six pedigrees showed IBD at consecutive markers from a single locus (table 2). Three Turkish kindreds (each with a single affected individual)—PB-022, PB-023, and PB-024—demonstrated IBD for two consecutive markers, *D15S650* and *D15S204*, and, in the case of PB-022 and PB-023, IBD for an additional proximal marker, *D15S814*. Furthermore, all three pedigrees shared the same allele (119/119) at *D15S650*, and PB-023 and PB-024 shared the 138 allele at *D15S204*. Since *D15S814* is outside the published critical interval (Bruford et al. 1997), we suggest that *BBS4* lies between *D15S650* and *D15S204*, a distance of 1.3 cM. Apart from PB-025, no other Turkish pedigrees were IBD for any markers tested on 15q23. A Pakistani family, PB-045, was exclusively IBD for all

**Table 2****Haplotypes of Consanguineous Pedigrees for Markers Encompassing the *BBS2* and *BBS4* Critical Regions**

Critical Region and Marker	PB-022	PB-023	PB-024	PB-025	PB-044	PB-045	PB-046
<i>BBS2</i> :							
<i>D15S204</i>	135/135	138/138	138/138	H	125/125	H	117/117
<i>D15S650</i>	119/119	119/119	119/119	123/123	135/135	H	135/135
<i>D15S814</i>	214/214	214/214	H	214/214	H	H	218/218
<i>D15S988</i>	H	H	H	H	H	H	268/268
<i>BBS4</i> :							
<i>D16S421</i>	H	H	H	H	H	212/212	212/212
<i>D16S400</i>	H	H	H	H	H	198/198	198/198
<i>D16S503</i>	H	H	H	H	H	304/304	294/294
<i>D16S408</i>	H	H	H	H	H	247/247	247/247

NOTE.—Where homozygous, haplotypes are shown as allele sizes in bp; H = heterozygous genotype.

four markers tested from the *BBS2* locus (16q21), whereas PB-044 was IBD for the two consecutive *BBS4* markers *D15S650* and *D15S204*. Although none of the alleles were common to the Turkish kindreds, loss of IBD at *D15S814* concurs with the Turkish interval. Unexpectedly, another Pakistani pedigree (PB-046) was IBD for all markers from both the *BBS2* and *BBS4* intervals. More importantly, however, we did not observe any conflicts in the critical interval as defined by haplotyping in the outbred and consanguineous cohorts. Instead, we have evidence of verification of the *BBS2* and *BBS4* critical intervals reported above and a potential reduction of the *BBS4* critical interval to 1.3 cM.

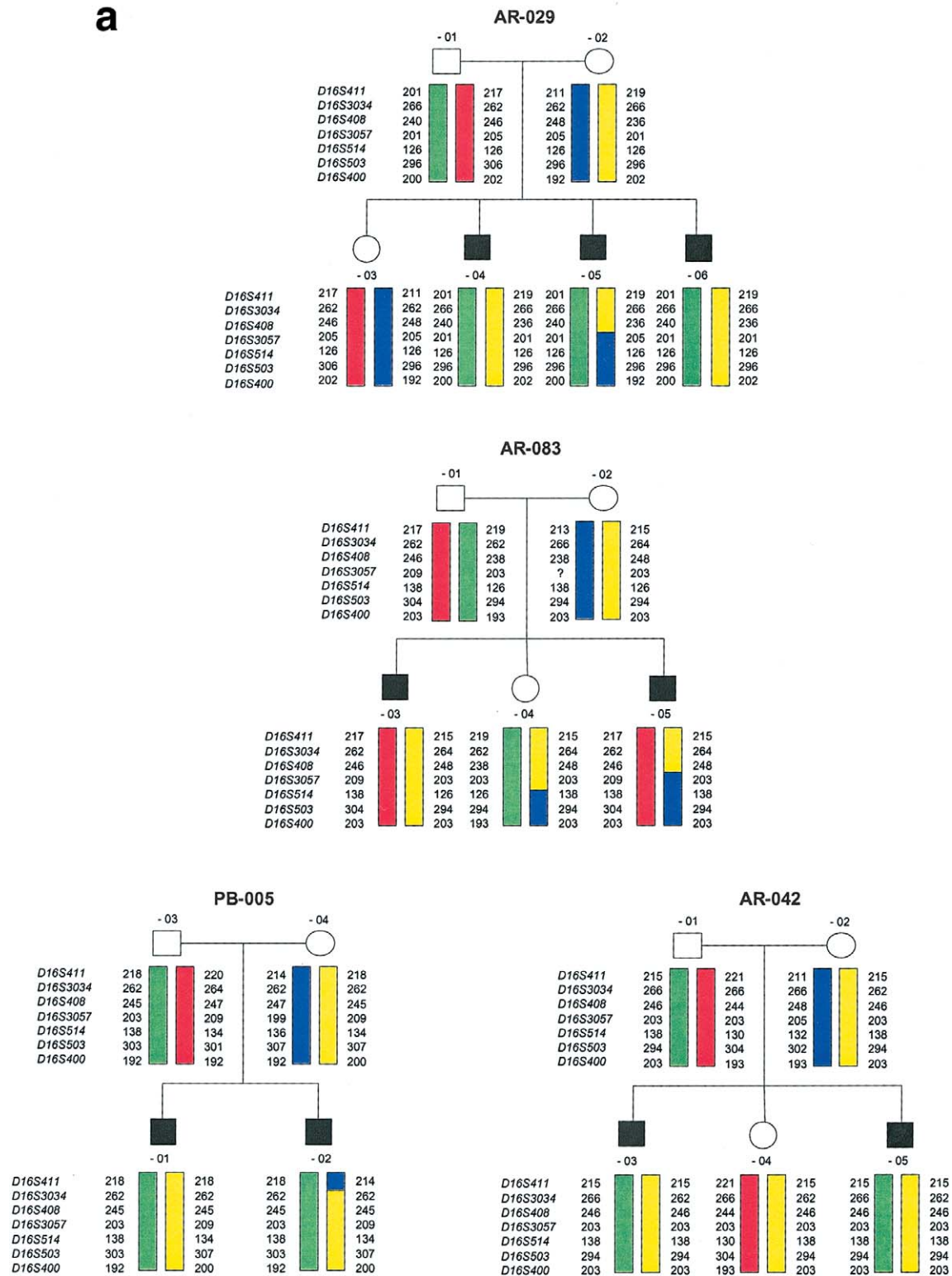
## Discussion

Substantial difficulties arise in the determination of linkage to any given gene locus in monogenic syndromes such as BBS, in which the disorder is rare, recessive, and genetically heterogeneous. We reported elsewhere the use of haplotype analyses to delineate the *BBS1* critical interval (Katsanis et al. 1999). The major contribution of that locus (40%–50%) to the syndrome allowed us to apply a haplotyping strategy because of the high probability that, in each pedigree of North American/European origin, the disorder mapped to *BBS1*. Here, we have addressed the much larger and more complicated question of the global genetics of BBS. This issue is substantially more challenging because of both the smaller contribution of each *BBS* locus and the fact that, in a substantial fraction of pedigrees, the disorder cannot be linked to any known locus. Despite these difficulties, we were able to identify families consistent with linkage to a single locus and thus propose refined boundaries for several critical intervals. In addition, the recent identification of *BBS6* allowed us to test the accuracy of this haplotype technique. Of the eight pedigrees in which we found mutations, seven were not associated previously

with any of the known loci through haplotype analysis, but were classified as “locus unknown.” This suggests that, although pedigrees are assigned to BBS loci according to a best-fitting haplotype–deduced model rather than absolute statistical criteria, these assignments are accurate, which in turn increases our confidence in the proposed delineation of the different *BBS* critical intervals.

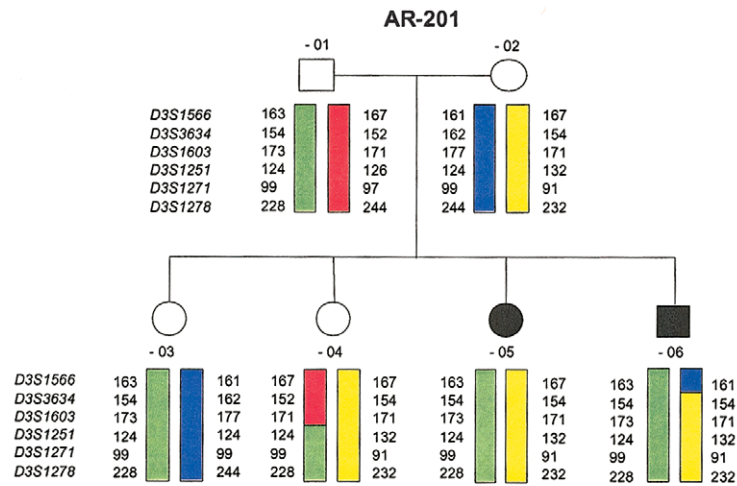
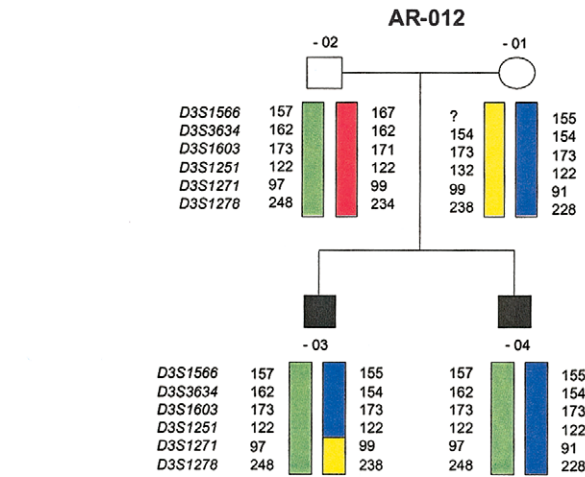
Newfoundland pedigree B14 was the only family in which a known *BBS6* mutation was observed but was expected to map to chromosome 16, by virtue of IBD across the *BBS2* critical interval. However, both genetic and mutational data suggest that the A242S alteration may not conform to a Mendelian model of disease transmission. First, we were unable to identify a second mutant allele. Second, and of greater importance, however, both affected and unaffected sibs had the same chromosome 20 haplotypes. One explanation for this might be that the A242S allele does not cause BBS but is a rare polymorphism. This conclusion would require that the H84Y component of the complex Amish MKKS allele is responsible for the phenotype. Alternatively, A242S may not be fully penetrant, although we have no evidence for such occurrence (there is no significant skewing of the numbers of affected sibs in our cohort). Finally, it is possible that A242S acts in conjunction with mutations at another locus, which would explain the family B14 haplotypes around *BBS2*, the inability to identify a substantial proportion of the second disease allele in patients with MKKS mutations, and the significant fraction of pedigrees for which we cannot assign the disorder to any locus. This model would not constitute true digenic inheritance—since there must be two mutations in *BBS2*, which, by themselves, are expected to cause disease—but rather a modifier effect for some MKKS mutations. Given that MKKS is a putative chaperone and may be a member of a multisubunit ring, such a finding may not be surprising, since mutations



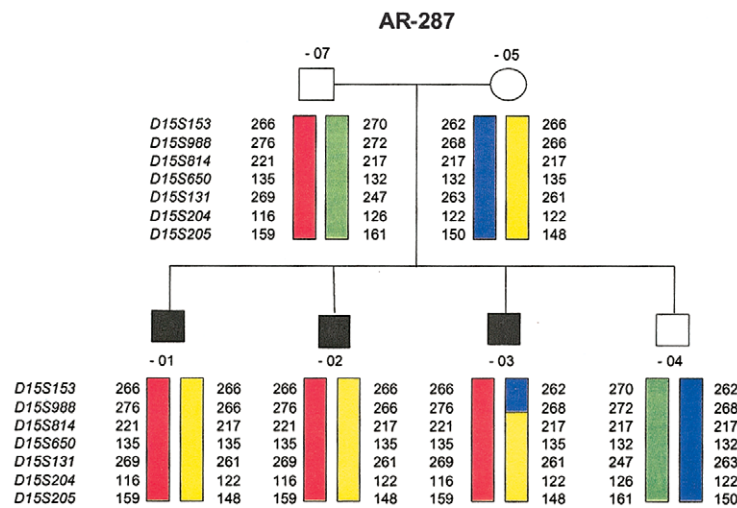


**Figure 3** Pedigrees and haplotypes consistent with linkage to chromosomes (a) 16q21 (*BBS2*), (b) 3p13 (*BBS3*), and (c) 15q23. Recombination events were observed in three pedigrees (AR-028, AR-083, and PB-005); when considered in combination with the proximal boundary of homozygosity (IBD) in AR-042, the new critical interval is defined by markers *D16S408* and *D16S3057*. Likewise, recombinations in AR-012 and AR-201 anchor the boundaries for *BBS3* at *D3S1603* and *D3S1271*. A single pedigree (AR-287) was consistent with linkage to *BBS4* on 15q23. A recombination event in individual -03 defines an overlap with the interval reported by Bruford et al. (1997) but does not narrow it further.

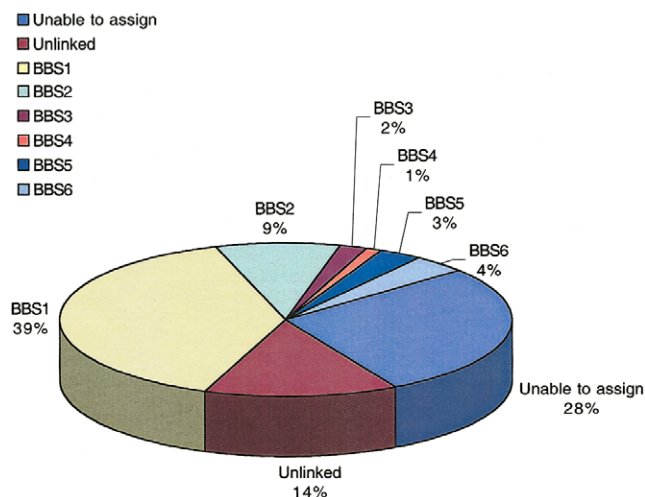
**b**



**c**







**Figure 4** Summary of the approximate relative distribution of each BBS locus in North American and European populations, based on a haplotype-inferred locus assignment of pedigrees. Between the present and the previous study (Katsanis et al. 1999), we obtained genotype data for 92 pedigrees, which we used to calculate relative contributions of each locus. Figures were then adjusted out of 96% to account for the 4% contribution of *BBS6*, which was ascertained by mutational analysis of a 163-patient cohort. In descending order of prevalence, *BBS1* > *BBS2* > *BBS6* > *BBS5* > *BBS3* > *BBS4*. Note that as many as 14% of all pedigrees did not show linkage of the disorder to any of the known loci; this, combined with the statistically significant exclusion of linkage to *BBS1*–*6* in families AR-707 and NF-B6, is highly suggestive of at least a seventh BBS locus.

in either multiple members of the ring and/or interacting proteins may further reduce the efficiency of binding and may result in more-severe phenotypes.

Pedigree NF-B14 does exhibit a severe phenotype, since the patients present with all the BBS features as well as early age at onset. However, given the substantial clinical heterogeneity of the syndrome, we must interpret these data with caution until additional *BBS* genes and a substantial number of pedigrees exhibiting this complex pattern of inheritance are identified. Intriguingly, two of the three pedigrees in which a single *BBS6* mutation was found and the pedigree structure was large enough to allow linkage studies (AR-259 and AR-301) are expected to show linkage of the disorder to chromosome 15. AR-259 is also homozygous for the distal portion of the *BBS4* critical region; in the third pedigree, AR-127, linkage was excluded from all known *BBS* loci.

In contrast to the *BBS6*-causing mutations reported elsewhere, most of which were frameshifts with an obvious deleterious effect on the protein, all mutations reported here are missense mutations. Therefore, it is more difficult to predict their effect on the MKKS molecule; nevertheless, most alterations are likely to affect either local polarity or the secondary or tertiary struc-

ture of the protein (table 1). Notably, three of the mutations lie in exon 6 of *MKKS*, which contradicts the hypothesis that exon 3 mutations are more severe and thus cause BBS rather than MKKS (Katsanis et al. 2000; Slavotinek et al. 2000).

Through a combination of haplotype analysis and homozygosity mapping, we assigned 14 North American/European families and six non-European families to *BBS2*–*5*. Thereby, the potential critical interval on chromosome 16q21 is reduced to a size amenable to positional cloning. We have also confirmed the *BBS5* locus on 2q31, previously reported in a single Newfoundland family (Young et al. 1999a), by adding three new pedigrees. Originally, *BBS3* on 3p13 was mapped by homozygosity techniques in a single, large Bedouin pedigree and was later confirmed in the same way in a single Newfoundland family (Sheffield et al. 1994; Young et al. 1998). Two of our North American families have haplotypes uniquely consistent with mapping to *BBS3*, and both serve to reduce the critical interval to 2 cM.

In light of the report by Bruford et al. (1997) in which 8 (35%) of 29 pedigrees from northern Europe showed linkage of the disorder to chromosome 15, it is surprising that only one of our European pedigrees is consistent with linkage to *BBS4*. However, five Turkish and Pakistani pedigrees showed IBD to *BBS4*, suggesting that there may be a more prevalent founder locus in these populations and that perhaps the best chance for identification of this gene may be through homozygosity mapping in Middle Eastern/Asian consanguineous kindreds.

Of interest, PB-046 was IBD for all markers in both the *BBS2* and *BBS4* intervals. We may interpret this in two ways: (1) This was a chance occurrence. Given that the parents are first cousins, they would be predicted to be IBD at two random but different loci 1/16 times and might have a homozygous putative alteration at either one of the two loci. However, dual incidences of IBD were not seen in any other consanguineous pedigree in this study. Alternatively, (2) this pedigree contains two homozygous mutations on different loci (pseudo-digenic inheritance), any one of which might be sufficient to lead to BBS by itself. As with NF-B14, where pseudo-digenic inheritance or a modifier effect of *BBS6* is speculated, we cannot attempt a genotype-phenotype correlation because of the substantial clinical variability of the syndrome.

Since the recent identification of *BBS6*, this is the first study to define the prevalence of mutations in *MKKS* among BBS pedigrees from North America and Europe. In contrast to Newfoundland, where *BBS6* accounts for the disorder in 40% of all families with BBS, our data show that only 4% of North American/European families with BBS have mutations in *MKKS*. We have also

accurately determined the overall distribution of *BBS* loci (fig. 4) by analysis of a large number of families from diverse populations. We have concluded that *BBS1* and *BBS2* account for the majority of cases among the North American/European population group, whereas *BBS4* is most prevalent among this population subset of Turks and Pakistanis. Of interest, the proportion of unassigned pedigrees in the latter cohort is very high (62%), suggesting that this population may still contain either a hitherto unidentified high-prevalence single locus or multiple minor loci.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, <http://gdbwww.gdb.org/>  
 Lupski lab home page, <http://www.imgen.bcm.tmc.edu/molgen/lupski/index.htm>  
 MIT Center for Genome Research, [http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys\\_map](http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map)  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for *BBS* [MIM 209900])

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